Naval Surface Warfare Center
Carderock Division
West Bethesda, MD 20817-5700

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Survivability, Structures, and Materials Department

Technical Report

Biofouling of Several Marine Diesel Fuels

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David Stamper, Michael Montgomery, and Robert Morris

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14. ABSTRACT

The US Navy is facing exposure to new fuels that may behave differently in the marine environment, in comparison with high sulfur diesel. This laboratory work has investigated the effects of biofouling and seawater exposure to high sulfur diesel, ultralow sulfur diesel, synthetic diesel, biodiesel, and hydrotreated renewable diesel fuels. Bulk chemical changes and differences in biofouing between the fuel were not detectable under the laboratory test conditions, but changes in fuel lubricity, controlled by traces of polar compounds in fuel, were detectable in several fuels by both physical and chemical testing. Machinery dependant upon fuel lubricity, such as fuel pumps and fuel injectors, may be at risk when new fuels enter use in the near future. Actual shipboard testing should be undertaken to determine the actual risk, however.

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Administrative Information

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Acknowledgements

The authors thank Sherry Williams at NAVAIR, Patuxent River Naval Air Station, for technical advice, and for arranging fuel samples for testing. We thank Jamie Sanders, also at Pax River, for physical lubricity testing by high frequency reciprocating rig (HFRR) and for total acid number analysis. We also thank Brittany Preston, Andrew Vorwald, and Arlene Thukral, all interns at Carderock West Bethesda, for sampling, measuring, and tending experiments.

Introduction

With environmental and geopolitical concerns over the sustainability of how we use petroleum fuels, the US Navy is undergoing changes in the liquid fuels used onboard ship. In the short term, ultra-low sulfur diesel (ULSD) fuel is slated to supplant "normal" low- and high-sulfur diesel (HSD) for marine uses, much as has occurred for domestic diesel fuel. The military specifications (MILSPECs) or requirements of ULSD fuel are currently being formulated (Williams and Chang 2008). Also in the short term, the US Navy faces the likelihood of exposure to biodiesel. Longer-term liquid fuel possibilities are renewable synthetic fuels created from biomass.

ULSD is refined including treatment with H_2 at high pressure and temperature, in the presence of catalysts, to reductively hydrogenate various compounds, particularly those containing S, N, or O. Aromatics and other unsaturated compounds may also be reduced, depending on the severity of this hydrotreatment (Harwell *et al.* 2003). The amounts of sulfurous and nitrogenous compounds in fuel are regulated because when they are burned, they produce sulfur dioxide (SO_2) and nitrogen oxides (NO_x) that combine with water to form acid rain (EIA 2001). Oxygen compounds are typically less abundant in petroleum, but are important for fuel lubricity (Hughes *et al.* 2002, 2003). Polar compounds, particularly long-chain fatty acids, are added back to ULSD to restore fuel lubricity.

Biodiesel is produced from oils and fats from plants and animals, *via* the transesterification of fatty acids with methanol to produce fatty acid methyl esters (FAME). Biodiesel has a lower specific and volumetric energy density than petroleum diesel, requiring more fuel for a given power and/or duration. Although biodiesel is polar and has excellent lubricity, the polarity of biodiesel causes other problems. Mushrush *et al.* (2005) describe problems with biodiesel exposure to seawater that include fuel instability, water separation problems, and filter plugging. For these reasons, the US Navy seeks to avoid exposure to biodiesel. This may be difficult, however, given the significant market penetration of biodiesel, and it is likely that US Navy ships will sometimes encounter biodiesel-blended fuel.

Other bio-derived fuels are being pursued, unlike the problematic FAME biodiesel, and in contrast with the Fisher-Tropsch processed synthetic diesel (F-T) under recent consideration. F-T fuel was dropped from US Navy consideration because of its large carbon footprint.

Biological material can be hydrotreated to produce a mixture of hydrocarbons which, with additional treatment, is converted into a low-carbon footprint isoparaffinic fuel. Hydrotreated renewable jet (HRJ) fuel derived from *Camelina sativa* and hydrotreated renewable diesel (HRD) fuel from algae are currently in production for large-scale testing by the US Navy. Their intended use will be as 50/50 blends with petroleum-derived fuel to allow for "drop-in" use in current machinery. For HRJ and HRD, as for ULSD, polar compounds must be added to improve lubricity.

ULSD, FAME biodiesel, synthetic and bio-derived diesel are qualitatively different from the fuels with which the U.S. Navy has had a long history. Notably, the new fuels have lower lubricity and/or lower (or zero) aromatics content. The chemical differences between the new fuels, especially in comparison with HSD, must be understood in order to maintain the reliability of Navy tactical vehicles and ships. Moreover, the biofouling potential and chemical and physical changes such biofouling may cause in the new fuels must be understood. This work seeks to gain such understanding. Fuels under investigation were ultra-low sulfur diesel (ULSD), synthetic diesel (Syn), biodiesel (Bio), and algae-derived diesel (HRD). HRD became available for testing late in 2010. Biodiesel was tested only at 5% by volume in ULSD. These fuels were all tested against the "old" high sulfur diesel (HSD) as the baseline fuel. To date, this project has demonstrated, under laboratory conditions, that 5% biodiesel supports higher biomass than the other fuels, and that fuel biofouling was able to degrade the lubricity improver additive. Shipboard conditions and samples have not yet been tested, however.

Materials and Methods

This work focuses on practical concerns over fuel biofouling under aerobic conditions. The two main issues are (1) the production of biomass and (2) the changes such biomass can produce in the fuel. Most testing involved a comparison of the "new" fuels (ULSD, 5% Bio in ULSD, Syn, and HRD (algae) against a baseline of the "old" HSD, performed in triplicate. Fuels were provided by NAVAIR Patuxent River (Sherry Williams). All exposures were in natural seawater collected from the DE shore (29 ppt salinity, pH adjusted to 8.0) at varying fuel-to-seawater proportions, typically 1:1 or 1:10. Some exposures involved adding NH_4Cl and K_2HPO_4 to 1 and 0.2 mM, respectively, intended as high-growth/worst-case conditions. Abiotic (negative) controls were poisoned by adding NaN_3 to 0.1% (1.0 $g \cdot L^{-1}$), and the effectiveness of this poisoning was confirmed by plating on marine agar (Difco, Detroit MI).

Fuel-Degrading Enrichment Culture

The experimental inoculum was derived from several sources, including an aqueous sample from the compensated fuel tank of a US Navy cruiser (USS Gettysburg – CG 64, 12/08 in Mayport, FL), water from Norfolk Naval Station, VA (2/09), and water and sediment from a commercial harbor, (Rudee Inlet, Virginia Beach, VA, 2/09). The Gettysburg sample was grown on HSD as the sole carbon and energy source: 500 ml natural seawater with 1.0 mM NH_4Cl and 0.2 mM K_2HPO_4 and 10 ml of HSD, with several transfers to fresh fuel and seawater over 10 weeks. Aliquots of this enrichment were stored frozen at -80°C. The water and sediment samples from Norfolk Naval Station and Rudee Inlet were similarly enriched and stored as aliquots. With the intention of using the same inoculum over time to improve repeatability, experiments were inoculated with the stored aliquots of both of these enrichments.

Biomass Measurements

Given the presence of filamentous fungi and bacteria (confirmed by microscopy), the production of acellular biomass in carbon-rich environments like fuel, and non-homogeneous distribution of the biomass, plate counts are completely inappropriate for biomass measurement in fuel and water mixtures. Instead of plate counts, total biomass was measured by total suspended solids dry weight (2540 D, Standard Methods for the Examination of Water and Wastewater).

Additional qualitative and semi-quantitative analysis of the biomass growing on the several fuels was by denaturing gradient gel electrophoretic (DGGE) analysis of polymerase chain reaction (PCR) amplified DNA. Additionally, DGGE fragments were cloned, sequenced, and compared against the sequence database for similarities to known bacterial and fungal sequences (Sequence ID). These molecular genetic analyses were provided by Molecular Insights, Inc (Rockford, TN). Community similarity analysis was done with Quantity One software (BioRad, Hercules, CA), with manual band location, Gaussian fitting of band intensity, and application of the UPGMA algorithm. Other algorithms produced similar results.

Fuel Toxicity

Early experiments suggested that HSD might support less biofouling than ULSD and Syn fuels, so the toxicity of HSD was tested by measuring its effect on oxygen consumption by the

enrichment culture. We used the BODTrak apparatus (Hach Co., Loveland, CO) to measure oxygen consumption over time as a function of pressure decrease in a sealed vessel. HSD was added in increasing amounts alone, or in combination with Syn or ULSD fuels to look for inhibition of respiration.

Chemical Changes in Fuels

Indirect chemical changes in the fuels were measured by respirometry, using the BODTrak apparatus (Hach Co., Loveland, CO) to measure oxygen depletion over time as a measure of biochemical oxygen demand (BOD). The BODTrak device was also used in previous fuel toxicity testing. Fuel lubricity was determined by high frequency reciprocating rig testing.

Changes in fuel composition were measured by gas chromatography/mass spectroscopy (GC/MS). Fuel samples from triplicate exposures were pooled for GC/MS and analyses were based on comparison to similarly-exposed abiotic (poisoned) controls. A low-factor Parallel Factor Analysis (PARAFAC) of the two GC/MS datasets was conducted to isolate the chemical constituents that changed during exposure with respect to the controls. The results of this analysis were passed to a compositional profiler that categorized the detected compounds that were either produced or consumed, with estimates of the relative percent changes in their concentrations.

Determining the expected rate of degradation of tall oil fatty acids (TOFA) lubricity improver involved $^{14}C_1$-linoleic acid additions to ULSD and HRD fuels (100 ppm final concentration). Rate of bacterial metabolism of this radiotracer was determined using mineralization to CO_2 and incorporation into bacterial biomass over a two week time series. Biotic (live) experiments were set up in triplicate, with a single acid-killed negative control, under different ratios and with and without added N and P. Bacterial growth was measured during the first 24 h of the incubations using the ^{3}H-leucine incorporation method (Smith and Azam 1992). Additional abiotic experiments were set up in triplicate to determine the initial partitioning of the ^{14}C-linoleic acid between each fuel and seawater (1:1 ratio with vortexing or shaking).

Results and Discussion

The several fuels tested for this report were qualitatively different, as shown in the chromatograms in Figure 1. HSD and ULSD each had a normal distribution of compounds, but the distribution of compounds for HSD was around a longer retention time, consistent with a higher average molecular weight. The FAME peak in the 5% Bio fuel was clearly evident against a backdrop of ULSD. The distribution of compounds in the Syn fuel was skewed to the left, with lower molecular weight compounds being more prevalent than higher molecular weight compounds. This distribution is to be expected, based on the particulars of the F-T reaction, based on stepwise carbonyl addition to the growing alkane chain.

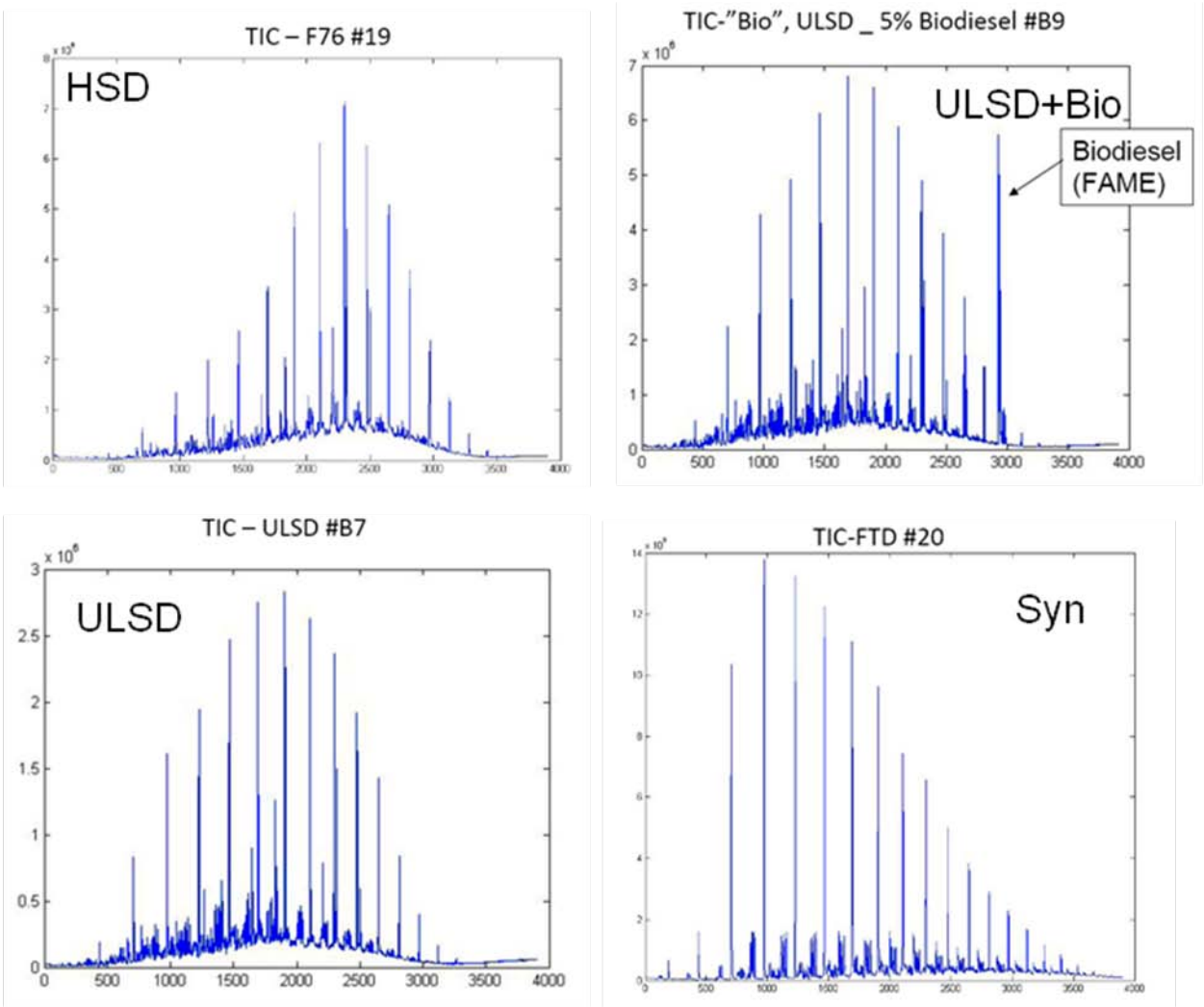

Figure 1. Total ion chromatograms (TICs) of HSD, 5% Bio, ULSD, and Syn fuels. TIC for for algae fuel not determined.

Fuel Toxicity

Experimentation with HSD alone and in the presence of Syn (Figure 2) found no inhibitory effect of HSD on the enrichment culture. Inhibition would have been demonstrated by less oxygen consumption with higher levels of HSD. The addition of more HSD increased the rate and extent of oxygen consumption, but with less than a 1:1 proportional response. This limitation is likely an effect of the very low solubility of most compounds in fuel. In any case, the lack of any measureable toxicity should not be surprising, since a major source of inoculum was from a compensated fuel tank and the enrichment culture was developed on HSD. Previous toxicity experimentation with a naïve-to-fuel inoculum found no toxicity from exposure to 400 µL HSD·L^{-1} (Stamper and Montgomery 2008). In fact, the wastewater-derived seed culture, although metabolizing glucose and alanine, acclimatized and began to metabolize the HSD within five days.

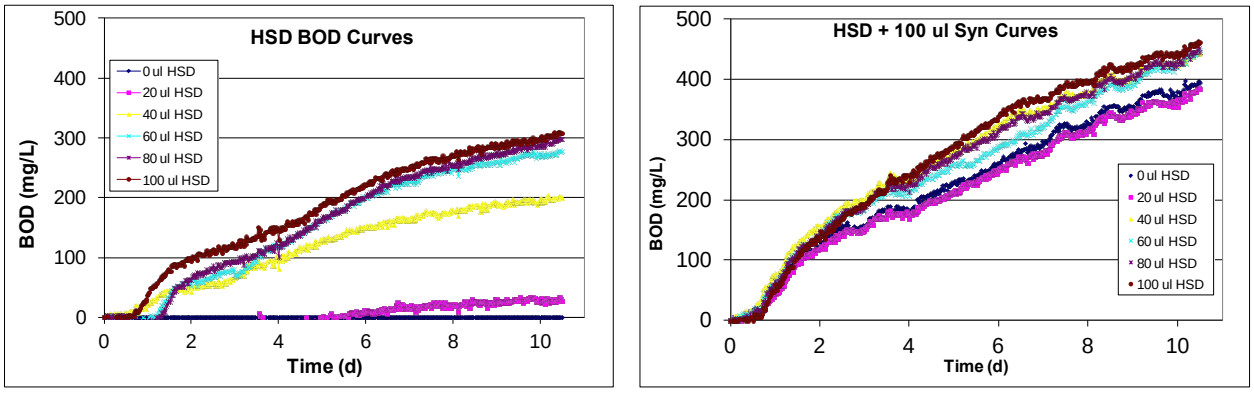

Figure 2. BOD response of the enrichment culture to exposure to HSD alone (left), up to 100 µL and to 100 µL Syn fuel with increasing amounts of HSD. The anomalous 20 µL HSD curve on the left figure was due to a leaking bottle.

Fuel Biofouling

For growth, microorganisms require energy, carbon, oxygen, nitrogen, phosphorus, sulfur, and various trace elements, all in useable form. A central point in this work is that compensated fuel tanks provide a carbon- and energy-rich environment for microorganisms, and that biofouling growth would not, ultimately, be limited by the availability of assimilable carbon and energy sources. The essentials supplied by the seawater are oxygen, nitrogen, phosphorus, sulfur, and trace elements.

In seawater, nitrogen and phosphorus are typically in short supply. Open ocean seawater averages 2.5 µM nitrogen and 0.07 µM phosphorus, with typical ranges of 0.4-36 µM and 0.03-1.9 µM, respectively (Riley and Skirrow 1965; Hill 1963). Near-shore and brackish waters would typically be higher. In any case, the 1 mM nitrogen and 0.2 mM phosphorus addition used in this experimentation is 400 times more nitrogen and 3 times more phosphorus than average seawater. These high concentrations were intended to stimulate "worst-case" conditions and effect changes in fuel composition that were large enough to measure.

Gross Biomass

Several biofouling experiments were unsuccessfully undertaken earlier in 2010, the problem finally determined to be due to the enrichment inoculum aliquots killed because of a freezer malfunction. Once this was realized, the inoculum was changed to an ongoing enrichment actively maintained on HSD since early 2009. This inoculum was demonstrated to be active by BODTrak respirometry and growth (Figures 3 and 4), and this enrichment will be used in future experimentation.

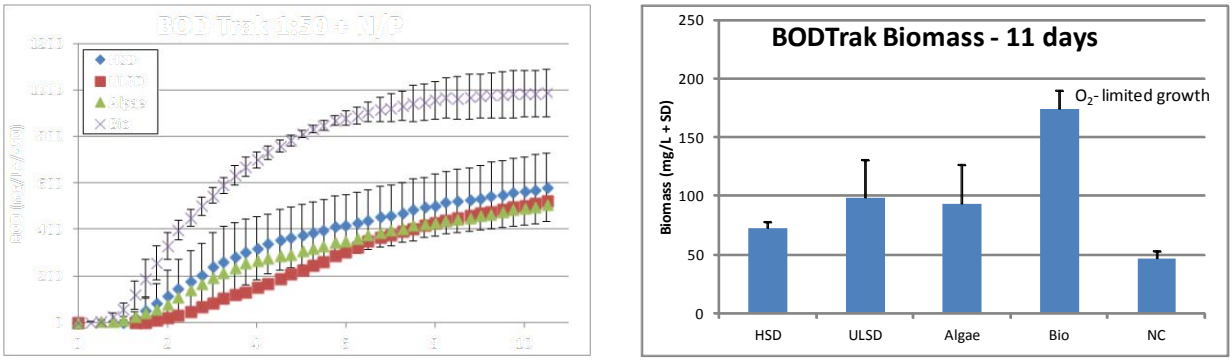

Figure 3. Respirometry (left) and biomass production (right) with HSD, ULSD, Algae, and 5% Bio fuels over 11 days. Fuel:SW::1:50, with N and P added to 1.0 and 0.2 mM, respectively and in triplicate. NC is the negative control for biomass production and normalizing the respirometry for BOD carryover in the inoculum. For the sake of clarity, not all error bars are shown on the respirometry plot (left). The capacity of this experiment was 700 mg·L^{-1} total BOD, which the 5% Bio exceeded.

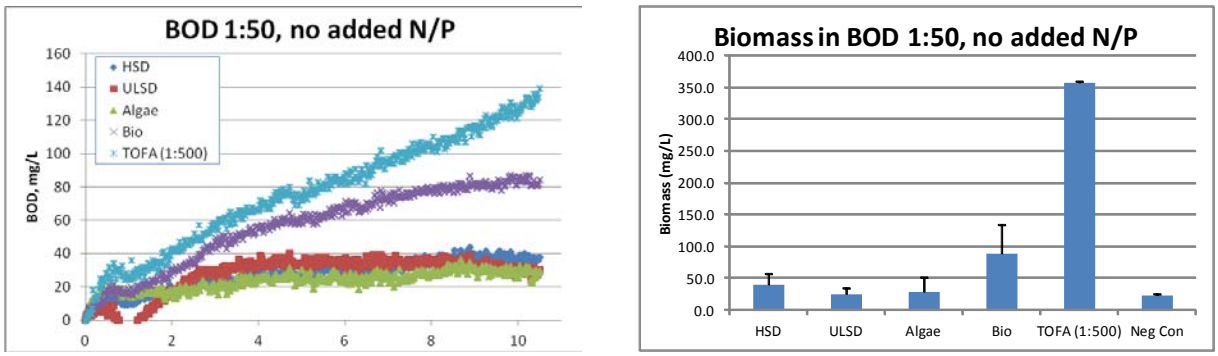

Figure 4. Respirometry (left) and biomass production (right) with HSD, ULSD, Algae, and 5% Bio fuels, and neat tall oil fatty acids (TOFA) over 11 days. Fuel:SW::1:50 and TOFA:SW::1:500, with no added N and P and in triplicate. Neg Con (right) is the negative control for biomass production and normalizing the respirometry for BOD carryover in the inoculum. Error bars for the respirometry (left) are not shown, but were approximately ±10%.

These results shown in Figures 3 and 4, although conducted at lower fuel:aqueous and with higher agitation, were consistent with prior results in that 5% Bio supported more biomass than the other fuels that otherwise supported approximately the same amount of biomass. Figure 4 shows the additional comparison of neat lubricity improver (TOFA) against the several fuels, without added N and P. TOFA, even though supplied at a lower fuel:aqueous ratio (1:500) than the several fuels, supported higher BOD and biomass; thus demonstrating the very high biodegradability of this lubricity improver additive relative to the bulk fuels. This was a predicted result early in this project, and additional data supporting the biodegradability of lubricity improver are shown below.

Biofouling Community Structure and Composition

Biomass from a 49 day exposure of HSD, ULSD, 5% Bio, and Syn fuels was analyzed for differences in microbial community structure, by PCR/DGGE. In DGGE, a band is usually (Wang and Wang, 1997) a single species and the band intensity is a semi-quantitative representation of that species' abundance. To be more precise, DGGE band intensity quantifies the proclivity of that species' DNA to be amplified under the current conditions (Polz and Cavanaugh, 1998). Still, PCR/DGGE is a widely accepted method for comparing communities; particularly those communities that are derived from the same source.

Figure 5 shows the 16S rDNA bacterial banding pattern and community similarity analysis for the microbial communities that developed on HSD, ULSD, 5% Bio, and Syn fuels, over 49 days. Figure 6 shows the equivalent for the 18S rDNA fungal banding pattern and community similarity. The HSD, ULSD, 5% Bio, and Syn fuels all support similarly rich and even communities of microbes and the communities supported by those four fuels were all quite similar to each other, in spite of chemical differences among them.

Considering the fact that the inoculum (originally enriched on HSD) for each of the fuels was the same, it is not surprising that the bacterial and fungal communities that developed over 49 days were similar. In both the bacterial and fungal communities, the Syn fuel was the out-group at 50-55% similarity to the other communities. Otherwise, the communities that developed on the other three fuels were more similar to each other. One small difference is that the 5% Bio was more similar to HSD in the bacterial communities (Figure 5), whereas the ULSD clustered more tightly with HSD for the fungal communities (Figure 6). The replicability of the PCR/DGGE methodology, in work with wastewater communities, was demonstrated at 90-95% similarity (Stamper *et al.* 2003). Considering this upper limit on similarity at 90-95% and a 49 day incubation in 4 different fuels, the overall similarities of 50-70% are quite high.

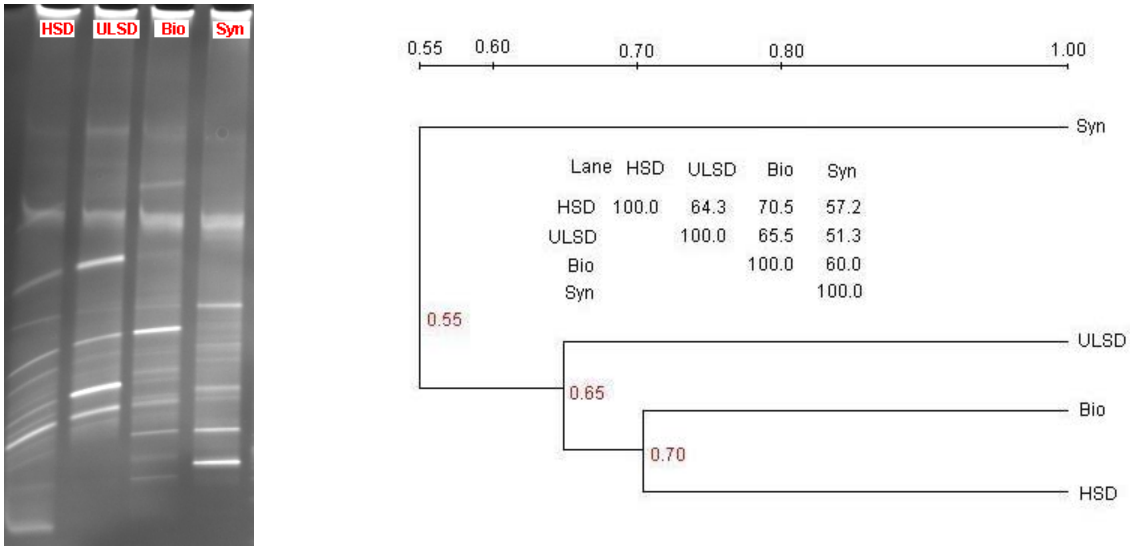

Figure 5. Bacterial DGGE banding pattern (left) and community similarity analysis (right) after 49 days growth on HSD, ULSD, 5% Bio, and Syn fuels. Primers were 341F(GC) and 907R, resulting in amplified fragments of approximately 560 bp.

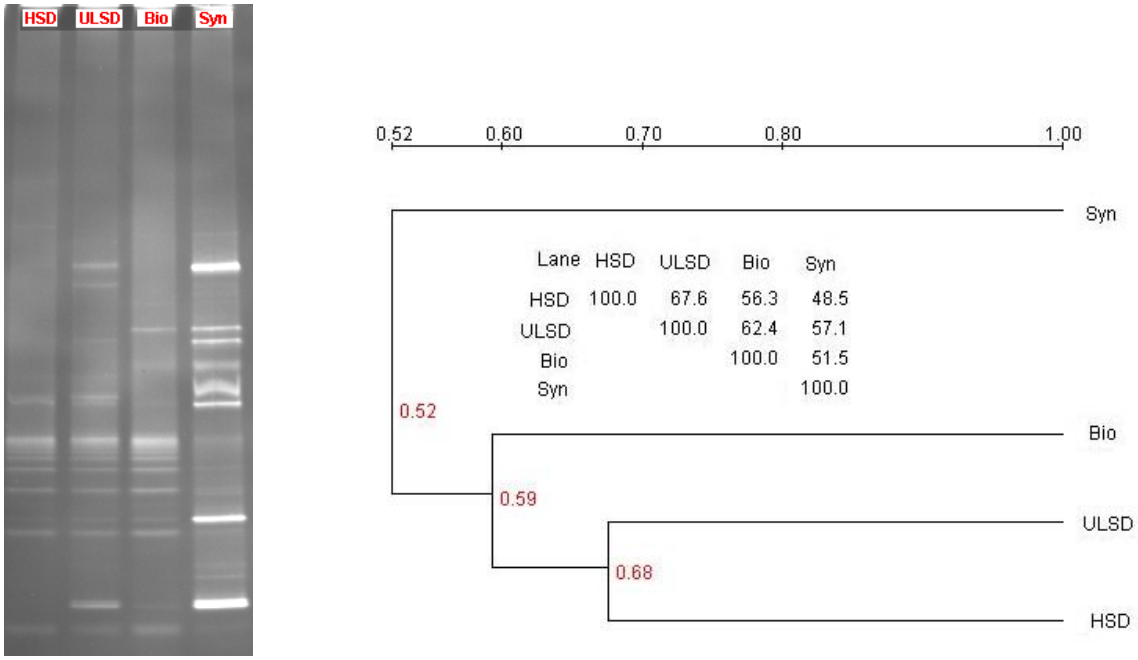

Figure 6. Fungal DGGE banding pattern (left) and community similarity analysis (right) after 49 days growth on HSD, ULSD, 5% Bio, and Syn fuels. Primers were U1F(GC) and U2R, resulting in amplified fragments of approximately 500 bp.

Table 1. Bacterial and fungal "species", evenness, and diversity as derived from the DGGE band count and intensities of Figures 5 and 6.

Fuel	Bacterial "Species"	Bacterial Evenness	Bacterial Diversity	Fungal "Species"	Fungal Evenness	Fungal Diversity
HSD	14	0.981	2.59	14	0.959	2.53
ULSD	11	0.969	2.32	14	0.968	2.55
5% Bio	15	0.958	2.59	14	0.972	2.56
Syn	13	0.944	2.42	20	0.958	2.87

Beyond the banding pattern similarity analyses, the band count and intensity can be used to derive ecological parameters of species abundance, evenness, and diversity, which are shown in Table 1. Abundance of "species" is the number of bands. Evenness and diversity are natural log functions of band intensity and band count. An evenness of 1.0 is perfect, with each "species" having equal abundance. The bacterial and fungal communities on all four fuels have similarly high richness, evenness, and diversity, with one exception. The high number of fungal "species"

in the Syn fuel is surprising and not easily explained. The Syn fuel is chemically simpler with no aromatics and fewer *n*- and *iso*-alkanes and was expected to support less diversity.

Fuel Chemistry

Bulk fuel chemistry changes have not yet been seen in the fuels tested so far. Chemical analyses out to 25 days' exposure have shown only small and noisy changes, as shown in Figure 7. Another sample set for chemical analyses out to a 49 day exposure (*i.e.* the same set for which the bacterial and fungal communities were analyzed) was apparently lost in the shuffle of the already-mentioned freezer meltdown. Determining that bulk fuel chemistry is not measurably changed by biofouling, at least out to 25 days under our test conditions suggests that bulk properties of the fuels are not going to be affected in the field.

Previously published information suggests what kinds of changes in the fuel can be expected. For example, shorter alkanes are more easily biodegraded than longer alkanes, largely solubility and kinetics effects, but there are also different genes involved in some cases (Hamamura et al. 200; Smits et al. 2002; van Beilen et al. 2002; Whyte et al. 1998). Branched alkanes, because of steric hindrance caused by the branching, are less easily biodegraded than *n*-alkanes, with highly-branched alkanes proving recalcitrant to biodegradation (Atlas & Bartha 1993; Leahy & Colwell 1990; Swannell et al. 1996). For petroleum-derived fuels, monocyclic aromatics are easier to biodegrade than polycyclics for reasons of solubility, kinetics, and the relative abundance of the necessary genes (Bregnard, et al. 1997; Kanaly & Harayama 2000; Ringelberg, et al. 2001; Swannell, et al. 1996). These cited studies were not in environments like a compensated fuel tank, so evaluating fuel chemistry changes is a future goal. The approach to date has essentially been to decrease the fuel:seawater ratio from 1:1 to 1:10 and increase exposure time, and then to look for chemical changes in the fuels. This approach has required a great deal of analytical effort to produce ambiguous results. Measuring the bulk chemical changes that biofouling causes and the time under which those changes occur are important for predicting possible problems using new fuels, and should be determined in future work.

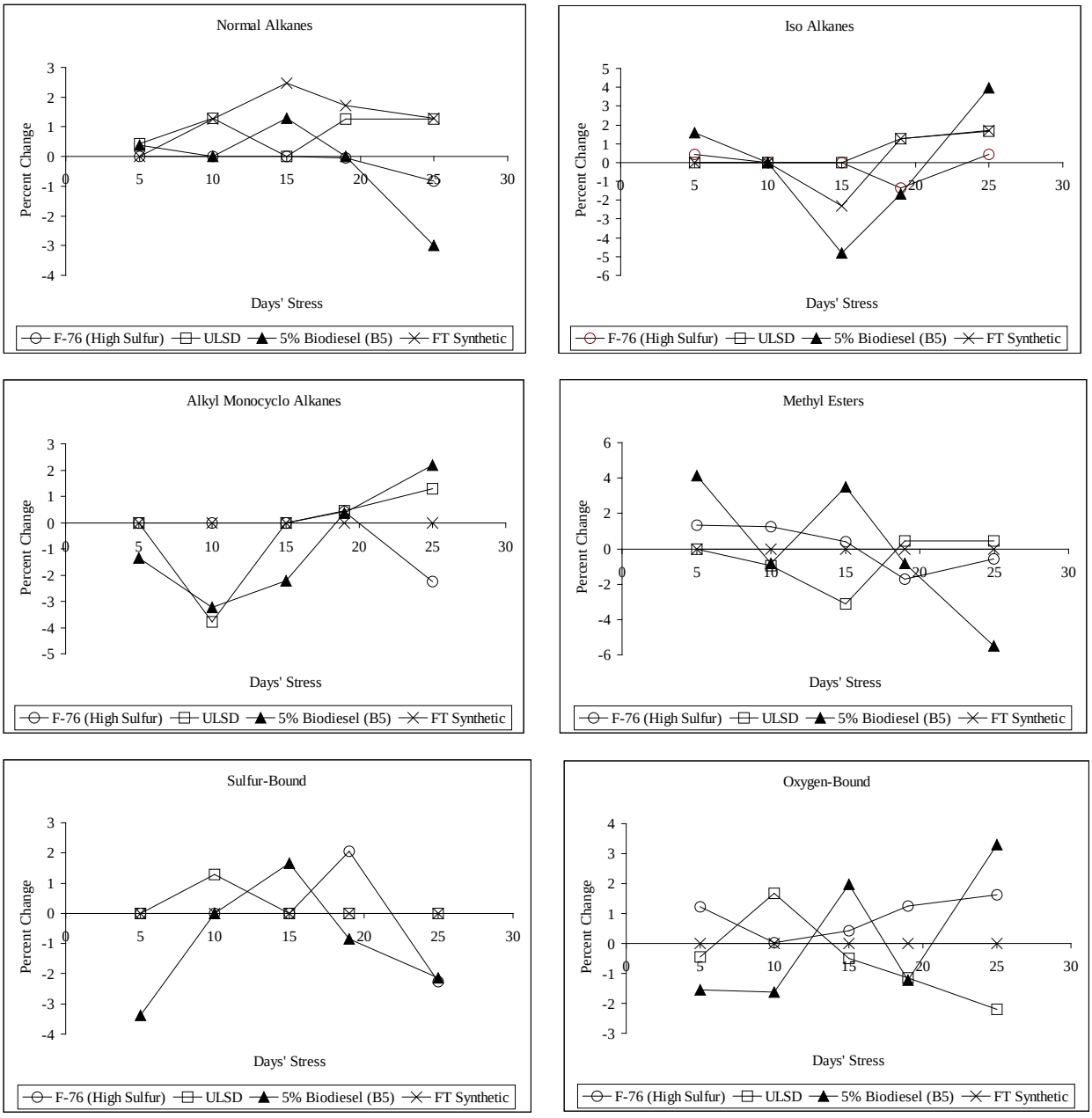

Figure 7. Noisy bulk chemical changes in F-76 (HSD), ULSD, 5% Bio, and FT Synthetic (Syn) fuels over 25 days exposure. All values are relative to a corresponding negative (poisoned) control. Categories are, left-to-right and reading down: *n*-alkanes, *iso*-alkanes, alkyl monocyclo-alkanes, methyl esters, sulfur-bound, and oxygen-bound.

The concern over trace chemical changes has focused on the lubricity improver required for hydrotreated fuels. Tall oil fatty acids (TOFA) was added to 100 ppm (vol:vol) to ULSD, Syn, and hydrotreated renewable (HRJ and HRD) fuels in all our experiments. The polarity of fatty

acids is responsible for their lubricity, as the polar carboxylic acid "head" of the molecule associates with metal or metal-oxide surfaces and provides boundary layer protection (Wadumesthrige 2005). Analogously, this polarity gives fatty acids much higher aqueous solubility than an equivalent *n*-alkane. Aqueous solubility is also very important in biodegradability and fatty acids are (theoretically) metabolized by every organism. Thus, the lubricity improver has higher solubility and higher and easier biodegradability than most (or possibly all) other compounds in fuels.

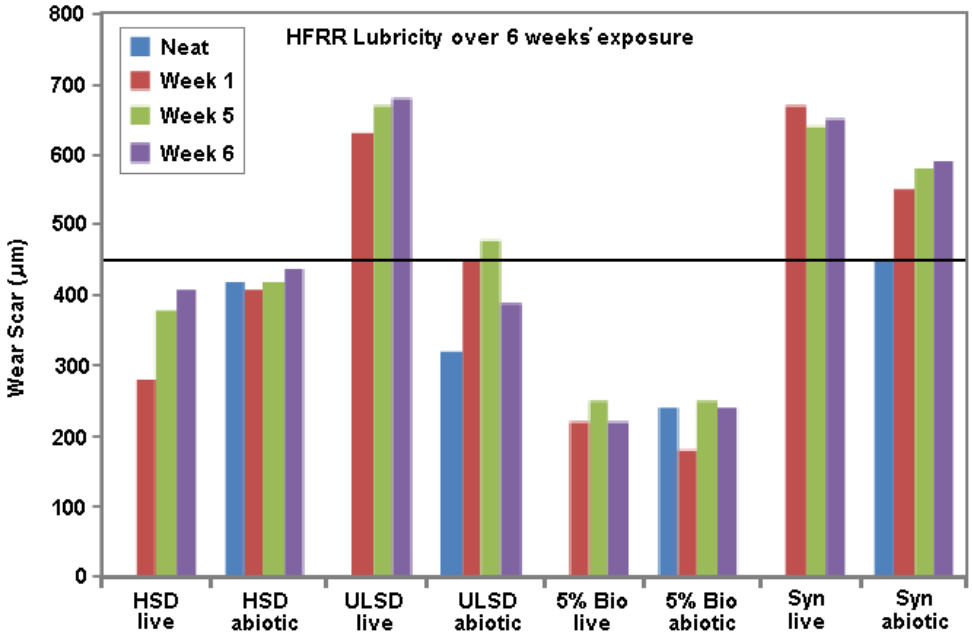

Figure 8. High frequency reciprocating rig (HFRR) test of fuel lubricity response to exposure to biofouling (live) and/or seawater (abiotic). The specification is for a wear scar ≤460 µm (line shown). The repeatability of the test is ±80 µm.

Physical lubricity of fuels was determined by high frequency reciprocating rig (HFRR). Fuels were exposed 1:10 to seawater alone (abiotic) or seawater plus biofouling (live) for up to 6 weeks. Lubricity was depleted upon exposure to seawater for Syn fuel, as well as from biofouling of ULSD and Syn fuels (Figure 8). In fact, even neat Syn fuel with 100 ppm TOFA had marginal lubricity, as did 2 of 3 seawater-exposed ULSD samples. HSD and 5% Bio remained within specifications for all exposures.

Since the physical testing demonstrated a problem with lubricity of some fuels upon exposure to seawater and biofouling, a sensitive chemical analytical approach was necessary to corroborate the results. The partitioning of lubricity improver (^{14}C-linoleic acid) in a 1:1::fuel:seawater is shown in Figure 9 for algae and ULSD fuels. TOFA, added to the fuels at 100 ppm, consists largely (82%) of two 18-carbon fatty acids: oleic and linoleic acid, each at 41% (Pine Chemicals Association 2001). Syn fuel was not tested in this round since this fuel is no longer under investigation for national policy reasons. These data were used to produce the fuel:seawater partitioning coefficients (K_{FsW}) for linoleic acid in these fuels, analogous to the octanol:water partitioning coefficient (K_{ow}) according to Equation 1, where [*LA_F] is the concentration of ^{14}C-linoleic acid in the fuel and [*LA_{sW}] is the concentration of ^{14}C-linoleic acid in the seawater.

$$K_{FsW}=\mathrm{Log}([*LA_F]/[*LA_{sW}]) \qquad \text{Equation 1}$$

The algae K_{FsW}= 2.26-2.29 and the ULSD K_{FsW} = 2.51-2.56, with lubricity improver in algae fuel being significantly more soluble than when associated with ULSD fuel. This 0.3-0.5% aqueous solubility is not likely enough to suggest that lubricity improver is depleted upon exposure to seawater, as suggested by the physical testing, but the K_{FsW} particular to Syn fuel remains unknown at this time.

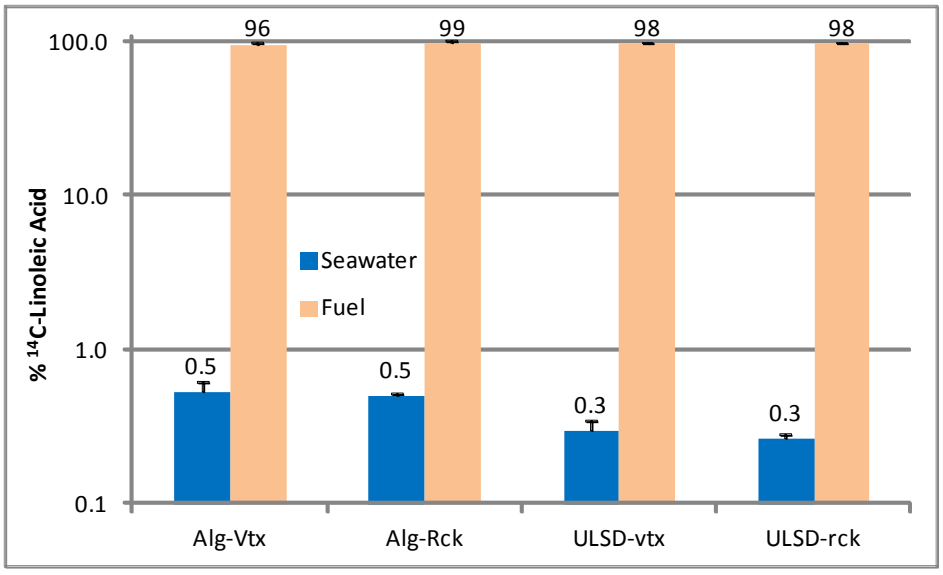

Figure 9. ^{14}C-linoleic acid partitioning after 30 min., in 1:1::fuel:seawater with algae and ULSD fuels containing 100 ppm TOFA, and with either rocking (rck) or vortexing (vtx).

The higher solubility of ^{14}C-linoleic acid is consistent with the differences in biological mineralization (complete oxidation to CO_2) of ^{14}C-linoleic acid shown in Figure 10. ^{14}C-linoleic acid in algae fuel was generally mineralized faster than from ULSD fuel. With no added N and P and a 1:1 ratio, 1.5-3.0% of the label was mineralized in 48 hours. This condition was intended to approximate "average" shipboard compensated fuel tanks. "Worst-case" conditions were simulated by adding N and P and/or increasing the aqueous proportion. These experiments find that lubricity improver is likely to be depleted upon exposure to biofouling in shipboard compensated fuel tanks, possibly at a rate of 0.8-8% per day, depending on the condition of the seawater and biofouling.

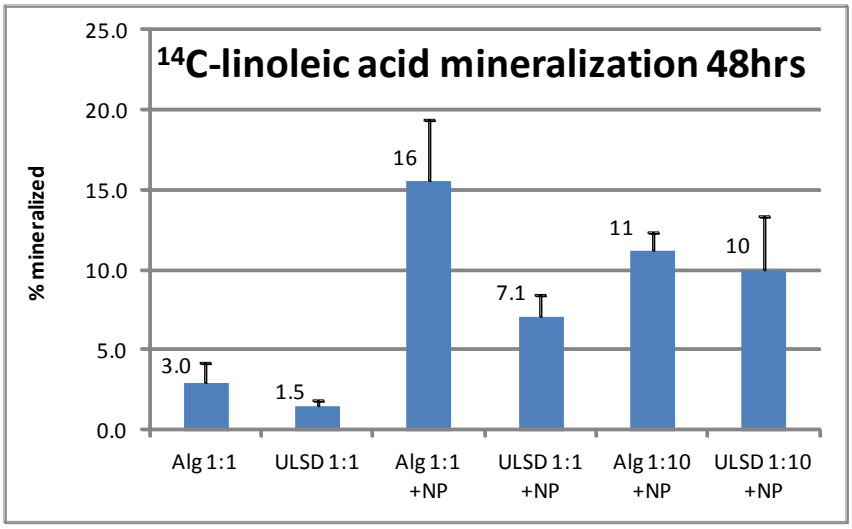

Figure 10. Mineralization of ^{14}C-linoleic acid in algae and ULSD fuels over 48 hours at different fuel:aqueous ratios and with and without added nitrogen and phosphorus to stimulate growth.

Summary

The US Navy is facing exposure to new fuels that may behave differently in the marine environment, in comparison with high sulfur diesel. This work has investigated the effects of biofouling and seawater exposure to high sulfur diesel, ultralow sulfur diesel, synthetic diesel, biodiesel, and hydrotreated renewable diesel fuels under laboratory conditions. Bulk chemical changes and differences in biofouing between the fuels were not detectable under the test conditions, but changes in fuel lubricity, controlled by traces of polar compounds in fuel, were

detectable in several fuels by both physical and chemical testing. Machinery dependant upon fuel lubricity, such as fuel pumps and fuel injectors, may be at risk when new fuels enter use in the near future. However, the extent to which these laboratory experiments apply to shipboard conditions remains unknown and should be confirmed by shipboard testing and evaluation.

Citations

Atlas, R.M., and R. Bartha. 1993. Microbial Ecology. Fundamental and Applications. Benjamin/Cummings Publishing Co. Inc., Redwood City, CA.

Bregnard, T.P-A., A. Häner, P. Höhener, and J. Zeyer. 1997. Anaerobic degradation of pristane in nitrate-reducing microcosms and enrichment cultures. Appl. Environ. Microbiol. 63:2077-2081.

Energy Information Administration. 2001. The Transition to Ultra-Low-Sulfur Diesel Fuel: Effects on Prices and Supply. Report #: SR-OIAF/2001-01, May 2001. Available at: http://www.eia.doe.gov/oiaf/servicerpt/ulsd/chapter3.html

Pine Chemicals Association, Inc. 2001. High production volume (HPV) chemical challenge program test plan for tall oil and related substances. Available at: http://www.epa.gov/hpv/pubs/summaries/tofars/c13056.pdf

Hamamura, N. C.M., Yeager, and D.J. Arp. 2001. Two distinct monooxygenases for alkane oxidation in *Nocardioides* sp. strain CF8. Appl. Environ. Microbiol 67:4992-4998.

Harwell, L., S. Thakkar, S. Polcar, R.E. Palmer, P.H. Desai. 2003. Study outlines optimum ULSD hydrotreater design. Oil Gas J. Jul/Aug 2003. Available at: https://portal.mustangeng.com/pls/portal30/docs/FOLDER/MUSTANGENG/TECHNICAL_ARTICLES_CONTENT/USDLHYDROTREATER.PDF

Hill, M. N. (Editor). 1963. The Sea. Ideas and Observations on Progress in the Study of the Seas. Vol. 2: Composition of Sea-Water. Comparative and Descriptive Oceanography. New York, London: Interscience Publishers, John Wiley & Sons Ltd

Hughes, J.M, G.W. Mushrush, and D.R. Hardy. 2002. Lubricity-enhancing properties of soy oil when used as a blending stock for middle distillate fuels. Ind. Eng. Chem.. Res. 41:1386-1388.

Hughes, J.M., G.W. Mushrush, and D.R. Hardy. 2003. The relationship between the base extractable species found in middle distillate fuel and lubricity. Energy Fuels 17:444-449.

Kanaly, R. and S. Harayama. 2000. Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria. J. Bacteriol. 182:2059-2067.

Leahy, J.G., and R.R. Colwell. 1990. Microbioal degradation of hydrocarbons in the environment. Microbiol. Rev. 54:305-315.

Mushrush, G.W, J.H. Wynne, C.T. Lloyd, H.D. Willauer, and D.R. Hardy. 2005. Incompatibility of recycled soy-derived biodiesels in marine environments. Ind. Eng. Chem. Res. 44:9969-9972.

Polz, M.F., and C.M. Cavanaugh. 1998. Bias in template-to-product ratios in multitemplate PCR. Appl. Environ. Microbiol. 64:3724-3730.

Riley, J. P. and Skirrow, G. (Editors) 1965. Chemical Oceanography. Vol. 1 & 2. – London and New York: Academic Press.

Ringelberg, D.B., J.W. Talley, E.J. Perkins, S.G. Tucker, R.G. Luthy, E.J. Bouwer, and H.L. Fredrickson. 2001. Succession of phenotypic, genotypic, and metabolic community characteristics during *in vitro* bioslurry treatment of polycyclic aromatic hydrocarbon-contaminated sediments. Appl. Environ. Microbiol. 67:1542-1550.

Smith, D.C., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51:201-213.

Smits, T.H.M., S.B. Balada, B. Witholt, and J.B. van Beilen. 2002. Functional analysis of alkane hydroxylases from gram-negative and gram-positive bacteria. J. Bacteriol. 184:1733-1742.

Stamper, D.M., M.T. Montgomery. 2008. Biological treatment and toxicity of low concentrations of oily wastewater (bilgewater). Can. J. Microbiol. 54:687-693.

Stamper, D.M., M. Walch, R.N. Jacobs. 2003. Bacterial population changes in a membrane bioreactor for graywater treatment monitored by denaturing gradient gel electrophoretic analysis of 16S rRNA gene fragments. Appl. Environ. Microbiol. 69(2):852-860.

Standard Methods for the Examination of Water and Wastewater, 17th Edition, 1989. Eds. L.S. Clesceri, A.E. Greenberg, R.R. Trussell, and M.A.H. Franson. American Public Health Association, Washington, DC.

Swannell, R.P.J., K. Lee, and M. McDonagh. 1996. Field evaluations of marine oil spill bioremediation. Microbiol Rev. 60:342-365.

van Beilen, J.B., M. Neuenshwander, T.H.M. Smits, S.B. Blalda, and B. Witholt. 2002. Rubredoxins involved in alkane oxidation. J. Bacteriol. 184:1722-1732.

Wadumesthrige, K., M. Ara, S.O. Salley, and K.Y.S. Ng. 2005. Investigation of lubricity characteristics of biodiesel in petroleum and synthetic fuel. Energy Fuels 23:2229-2234.

Walker, J.D., and R.R. Colwell. 1974. Microbial petroleum degradation: use of mixed hydrocarbon substrates. Appl. Environ. Microbiol. 27: 1053-1060.

Wang, G.C.-Y., and Y. Wang. 1997. Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16SrRNA genes from mixed bacterial genomes. Appl. Environ. Microbiol. 63:4645-4650.

Williams, S., and P. Chang. 2008. Personal communication on F-76/DFM lubricity and future MILSPEC. NAVAIR Fuel Group. 3/27/08.

Whyte, L.G., J. Hawari, E. Zhou, L. Bourbonniere, W.E. Inniss, and C.W. Greer. 1998. Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. Appl. Environ. Microbiol. 64:2578-2584.


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